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Deduced Polypeptides Encoded by the *Bacillus subtilis sacU* Locus Share Homology with Two-Component Sensor-Regulator Systems

FRANK KUNST,[1] MICHEL DEBARBOUILLE,[1] TAREK MSADEK,[1] MICHAEL YOUNG,[2,3] CATHERINE MAUEL,[3] DIMITRI KARAMATA,[3] ANDRE KLIER,[1] GEORGES RAPOPORT,[1]* AND RAYMOND DEDONDER[1]

Unité de Biochimie Microbienne, Institut Pasteur, 25, rue du Docteur Roux, 75724 Paris Cedex 15, France[1]; Department of Botany and Microbiology, University College of Wales, Aberystwyth SY23 3DA, United Kingdom[2]; and Institut de Génétique et de Biologie Microbienne, 1005 Lausanne, Switzerland[3]

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The *sacU* locus has been cloned by using two independent strategies, and the presence of two open reading frames was deduced from the nucleotide sequence. Open reading frame 1 encodes a 45,000-dalton polypeptide that is similar to the products of the *Salmonella typhimurium cheA* and *Escherichia coli cpxA* genes, which act as sensory transducers. Open reading frame 2 encodes a 26,000-dalton polypeptide that is similar to a family of transcriptional activators, including the products of the *Bacillus subtilis spo0A* and *spo0F* and the *E. coli ompR* and *dye* genes. These similarities suggest that the products of the *B. subtilis sacU* locus form a sensor-transducer couple, which functions to relay information about specific environmental changes to the transcription apparatus.

The expression of a class of structural genes encoding degradative enzymes in *Bacillus subtilis* is controlled by regulatory genes unlinked to the target genes. The production of several secreted enzymes, i.e., levansucrase, serine and neutral proteases, α-amylase, β-glucanase(s), xylanase (2, 4, 5, 24, 26), and one intracellular enzyme, serine protease (40), is affected by the *sacU* and *sacQ* regulatory genes. These genes have been identified as the sites of chromosomal mutations leading to levels of production of degradative enzymes that are either increased [referred to as *sacU*(Hy) *sacQ*(Hy)] or decreased (*sacU*) compared with that of the reference strain *B. subtilis* 168 (*sacU*$^+$ *sacQ*$^+$). The pleiotropic phenotype of *sacU*(Hy) mutants also includes the absence of flagella, low transformation efficiency, and altered control of sporulation (24). A similar phenotype was observed for a *sacQ*(Hy) mutant (24).

Little is known about *sacU*- and *sacQ*-mediated control at the molecular level, except that it apparently involves activation of transcription (44). An analysis was made of the upstream regions of two target genes, encoding levansucrase (*sacB*) and serine protease (*aprE*). Regions essential for transcriptional activation by *sacU* or *sacQ* were located between positions −117 and −96 and positions −164 and −141 with respect to the transcriptional start sites of *sacB* and *aprE*, respectively (4, 16, 17, 23), but precise target sequences have not yet been identified. The *sacQ* gene has been cloned and shown to encode a 46-amino-acid polypeptide (2, 51). Another gene, *prtR*, encoding a small polypeptide which affects degradative enzyme production has also been identified (31, 47, 52).

In this paper the cloning of the *sacU* locus is reported together with an analysis of its nucleotide sequence.

MATERIALS AND METHODS

Strains, phages, and plasmids. The strains of *B. subtilis* are listed in Table 1. *E. coli* TG1 [K-12 (Δ*lac-proAB*) *supE thi hsdD5* F′ *traD36 proA*$^+$ *proB*$^+$ *lacI*^{q} *lacZ* ΔM15] (T. J. Gibson, Ph.D thesis, University of Cambridge, 1984) was used as the host for M13mp18 or M13mp19. *Escherichia coli* P2392, which is lysogenic for P2 (F$^-$ *hsdR514 supE44 supF58 lacY1 galK2 galT22 metB1 trpR55*), was used as indicator organism for propagating recombinant EMBL3 phages. Packaging mixtures were obtained from strains BHB2688 and BHB2690 (20).

The plasmids pHV1431, pHV1432, and pHV1436 were obtained from L. Jannière and S. D. Ehrlich (submitted for publication). Plasmid pHV1431 (10.8 kilobases [kb]; Cm^r) contains a replication origin from plasmid pAMβ1 (25) and is maintained at high copy number in *B. subtilis*. Plasmid pHV1432 is a deleted derivative of pHV1431 in which one of the two *Eco*RI sites was eliminated. Plasmid pHV1431d is an 8.3-kb derivative of pHV1431 which arose by spontaneous deletion in *E. coli*, eliminating a DNA fragment containing the two *Eco*RI sites of pHV1431. Plasmid pHV1436 (8.7 kb; Cm^r) contains a replication origin from plasmid pTB19 (21) and is maintained at low copy number in *B. subtilis*. Plasmids pBU2, pBU3, pBU14, pBU16, pBU100, pBU101, pBU102, and pBU103 are described below. They were introduced into the *recE4* strain 1A510, for maintenance and preparative plasmid extraction, and into the *sacU* mutant QB254, to test their capacity to restore levansucrase synthesis. Plasmid pAH101 (21 kb: Ap^r Cm^r) was a generous gift from G. R. Stewart, University of Kansas. It contains about 11 kb of *B. subtilis* DNA flanking a Tn*917* insertion in the vicinity of the *tag-3* (*rodC1*) marker (38). This segment was cloned in *E. coli* by A. L. Honeyman and G. C. Stewart (Abstr. Annu. Meet. Am. Soc. Microbiol. 1985, H 91, p. 123) after plasmid pTV21Δ2 (53) was integrated into the transposon.

The phage lambda replacement vector EMBL3 (13) was used for construction of a gene bank of *B. subtilis* DNA. Phages λ54 and λ63, isolated from this bank, are described below.

Media and qualitative tests. *E. coli* was grown in L broth (10 g of tryptone, 5 g of yeast extract, and 5 g of NaCl per liter) and *B. subtilis* in Penassay antibiotic medium 3 (Difco Laboratories, Detroit, Mich.) or MMCH medium, which consists of 60 mM K_2HPO_4, 44 mM KH_2PO_4, 15 mM $(NH_4)_2SO_4$, 3 mM trisodium citrate, 2 mM $MgSO_4$, 0.01 mM $MnCl_2$, 22 mg of ferric ammonium citrate per liter, 0.05%

* Corresponding author.

TABLE 1. Strains of *B. subtilis*

Strain	Genotype	Relevant phenotype	Origin or reference
168	*trpC2*	Lvs$^+$ Prt$^+$	Laboratory stock
L5047	*trpC2 pheA1 his-35 purA16 metB5*	Lvs$^+$ Prt$^+$	Laboratory stock
QB254	*trpC2 hisA1 sacA321 sacU42*	Lvs$^-$ Prt$^-$	Laboratory stock
QB136	*trpC2 leuA8 sacU32*	Lvs(Hy) Prt(Hy)	24
L5065	*trpC2 pheA1 his-35 purA16 sacU32*	Lvs(Hy) Prt(Hy)	QB136 $\xrightarrow{\text{tfm}^a}$ L5047
1A510	*leuA8 arg-15 thrA5 recE4 stp*	Lvs$^+$ Prt$^+$	36
BD1238	*hisA1 leu metB5 com-524*::Tn*917lacZ*	Lvs$^+$ Prt$^+$	1, 15
QB4210	*trpC2 sacU350*::*erm-lacZ*	Lvs$^-$ Prt$^-$	This study
QB4222	*trpC2 sacU351*::*aphA3*	Lvs$^-$ Prt$^-$	This study

[a] tfm, Constructed by transformation.

casein hydrolysate, and 100 mg of auxotrophic requirements per liter. L plates or SP plates were prepared by the addition of 17 g of Bacto-Agar (Difco) per liter to L broth or SP medium (2, 26).

Colonies containing the *sacU*$^+$ (Prt$^+$) and *sacU32* [Prt (Hy)] marker were distinguished by plating on TS medium (22) overlayered with 2.5% (wt/vol) casein, on which they formed small and large halos, respectively. Colonies producing levans (Lvs$^+$ phenotype) were identified on ST plates containing 20 g of filter-sterilized sucrose (BDH, Poole, United Kingdom), 1 g of tryptone (Difco), and 17 g of purified agar (Difco) per liter, 13 mM KCl, 1 mM $MgSO_4$, and auxotrophic requirements (100 mg/liter). The mucoid aspect of levans surrounding Lvs$^+$ colonies on ST plates distinguished them from Lvs$^-$ colonies. SP or ST plates containing 5 μg of chloramphenicol per ml, 5 μg of kanamycin per ml, or 1 μg of erythromycin plus 25 μg of lincomycin per ml were used for selecting recombinants resistant to chloramphenicol (Cm^r), kanamycin (Km^r), or erythromycin (Em^r).

DNA manipulations and cloning procedures. Standard procedures were employed for extracting plasmids from *E. coli* (28) and *B. subtilis* (2) and for extracting DNA from lambda phages (14, 28). Restriction enzymes, T4 DNA polymerase, Klenow polymerase, and T4 DNA ligase were used according to the manufacturers' recommendations. DNA fragments were recovered from agarose gels by using either electroelution or Gene Clean (Bio 101, La Jolla, Calif.).

Ligations were performed at high DNA concentrations (50 to 100 μg/ml) when mixtures were used for direct transformation of *B. subtilis* competent cells. For the construction of plasmid pBU102 (see Results), two DNA fragments were ligated: *Eco*RI-linearized pBU16, made blunt by Klenow polymerase, and a 4.5-kb *Sma*I-*Kpn*I fragment containing the *lacZ* and *erm* genes from pTV32 (37), made blunt by T4 DNA polymerase. Equimolar amounts of the two fragments were ligated and used for transformation of *B. subtilis* 168.

For construction of the gene bank of *B.* subtilis DNA in EMBL3, phage DNA was digested successively with *Bam*HI and *Eco*RI to prevent religation of the middle fragment to the arms. *B. subtilis* 168 chromosomal DNA was partially digested with *Sau*3A, and fragments in the size range of 12 to 24 kb were purified from agarose and ligated to the phage arms. Packaged recombinant phage were plated on lawns of the indicator strain P2392, and plaques were screened by hybridization on nitrocellulose filters (28).

Transformation of competent cells. Previously described methods for transformation of *B. subtilis* were followed (3, 32). Competent cells were incubated with plasmid DNA for 20 min at 37°C. Yeast extract (5 mg/ml), casein hydrolyzate (5 mg/ml), and auxotrophic requirements (50 μg/ml) were added at the indicated final concentrations, and incubation with shaking at 37°C was continued with sublethal antibiotic concentrations (0.5 μg of chloramphenicol per ml for 45 min, 0.2 μg of kanamycin per ml for 45 min, 0.15 μg of erythromycin per ml for 90 min) before plating on selective SP medium containing chloramphenicol, kanamycin, or erythromycin-lincomycin.

For recipient strains containing the *sacU32* marker, the transformation procedure described by Karamata and Gross (22) was used with the following modifications. Growth in SPIZ I medium was at 37°C, and cultures were incubated for 70 min after the end of the exponential growth phase before 10-fold dilution in SPIZ II medium.

Assay of levansucrase activity. Appropriate dilutions of MMCH culture supernatants were incubated at 37°C with 10% sucrose–50 mM potassium phosphate buffer (pH 6.0) in a final volume of 1 ml. Reactions were stopped by boiling, and glucose was measured with the GOD-Perid reagent (Boehringer Mannheim Biochemicals, Indianapolis, Ind.). One unit corresponds to 1 μmol of glucose produced per min. The total amount of protein was estimated from the optical density of the culture.

DNA sequencing. Nucleotide sequencing was carried out by using the dideoxy-chain termination method (41) and modified T7 polymerase (sequenase) (U.S.B., Cleveland, Ohio). Overlapping deletions were obtained by the method of Dale et al. (7) with a cyclone kit purchased from IBI (New Haven, Conn.).

In vitro transcription and translation. Covalently closed circular plasmids were used as templates for a prokaryotic coupled transcription-translation system as recommended by the manufacturer (Amersham Corp., Arlington Heights, Ill.). Proteins were labeled with [^{35}S]methionine (specific activity, >39 TBq/mmol), separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (exponential gradient of 7.5 to 15% acrylamide and 0.2 to 0.4% bisacrylamide), and visualized by autoradiography.

RESULTS

Cloning of the *sacU* locus of *B. subtilis*. Two independent strategies proved successful for isolating the *sacU* locus of *B. subtilis*. For the first, a Tn*917* insertion, located near *tag-3* (*rodC1*), was used to clone the *sacU* locus by chromosome walking. From a sample of about 1,600 plaques of recombinant EMBL3 phages, 14 hybridized with the *B. subtilis* sequences in plasmid pAH101. The phage extending furthest in the direction of *sacU* (λ63) was identified by physical analysis of its DNA. A 3.5-kb *Eco*RI-*Sal*I fragment, forming the *sacU*-proximal extremity of the insert in λ63 (Fig. 1), was used to rescreen the original sample of plaques. This second

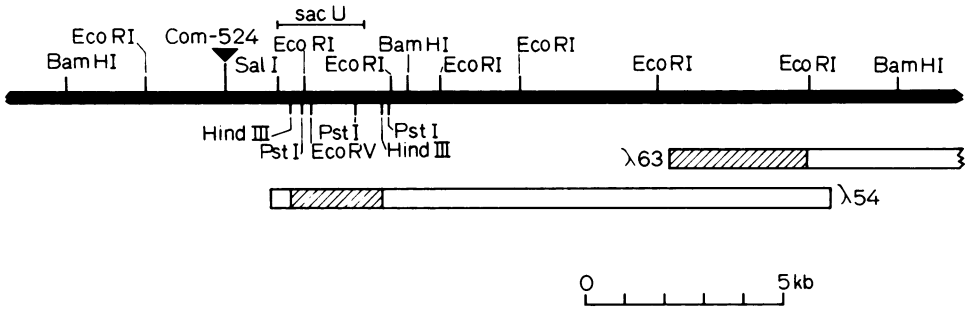


FIG. 1. Restriction map of the DNA segments cloned in phages λ54 and λ63. The bacterial chromosome is represented as a bold line. The positions of *Eco*RI, *Bam*HI, and *Sal*I sites were determined by physical analysis of phage DNA. The positions of *Hin*dIII, *Pst*I and *Eco*RV sites in the vicinity of *sacU* were determined by Southern hybridization. The site of the Tn*917lacZ* insertion corresponding to the *com-524* mutation is indicated; the Tn*917* insertion close to *tag-3* (*rodC1*) is about 12 kb to the right of the rightmost *Bam*HI site. The segments of the λ phages that were used as hybridization probes are indicated (▨).

step revealed four additional hybridizing plaques, from one of which phage λ54 was isolated (Figure 1).

Since extensive screening of the SacU$^+$ phenotype is not easy and since competent cells are rare, we devised a procedure to select these cells by using the selective marker *pheA1*. DNA extracted from phage λ54 was mixed in a 1:10 ratio with that from a phage with transforming activity against *pheA1* strains of *B. subtilis*. This phage had previously been isolated from the λ Charon 4A bank of Ferrari et al. (11) and was provided by M. Sargent. The above mixture was employed to transform the *B. subtilis sacU*(Hy) mutant L5065. About 12% (13 of 113) of the Phe$^+$ transformants simultaneously acquired the donor *sacU*$^+$ (Prt$^+$) allele, indicating that phage λ54 contains at least part of the *sacU* locus. The absence of *sacU*$^+$ transformants in similar crosses with λ63 donor DNA suggested that the *sacU* locus lies within the DNA segment that is unique to λ54.

A more detailed restriction map of the λ63-distal end of the insert in λ54 (Fig. 1), constructed by hybridization to appropriately digested samples of strain 168 chromosomal DNA, revealed that this region corresponded to that independently isolated in recombinant plasmids (see below).

For the second approach, a Tn*917lacZ* insertion (37, 43) that created the *com-524* mutation (1, 15) was used to clone the *sacU* locus directly in plasmids. This marker was shown by Dubnau and co-workers (1, 15) to be linked to *hisA1* by PBS1 transduction as was shown before by us in the case of *sacU* (45). Therefore, we thought that *com-524* may be closely linked to *sacU*. We measured the linkage between *com-524* and *sacU* by transformation of the *sacU* mutant QB254 with a nonsaturating concentration of BD1238 DNA. Fifty percent of the selected Em^r transformants became *sacU*$^+$ (Lvs$^+$). This close linkage was very useful for cloning the *sacU* locus. A unique *Bam*HI site located near one extremity of Tn*917lacZ* allowed us to clone DNA sequences adjacent to the *com-524* mutation by inserting *Bam*HI fragments of BD1238 chromosomal DNA into plasmid pHV1436 and selecting for Em^r by using *B. subtilis* 168 or 1A510 as the recipient. Plasmid pBU2 (Cm^r Em^r) was obtained in this way. It contains a 13.5-kb *Bam*HI fragment of BD1238 DNA. Plasmid pBU2 contained at least part of the *sacU* locus, since it conferred a Lvs$^+$ phenotype on strain QB254.

The *Bam*HI fragment from pBU2, containing Tn*917lacZ* and *sacU* DNA sequences, was transferred to a vector

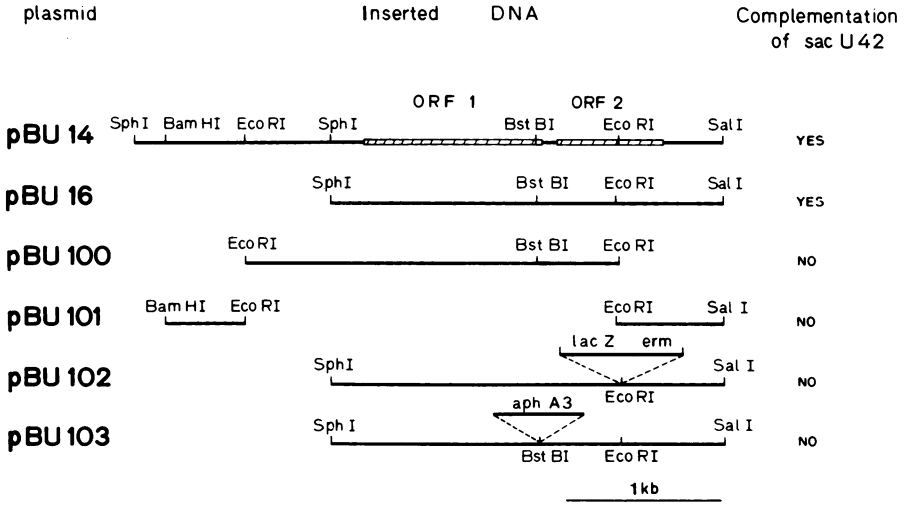
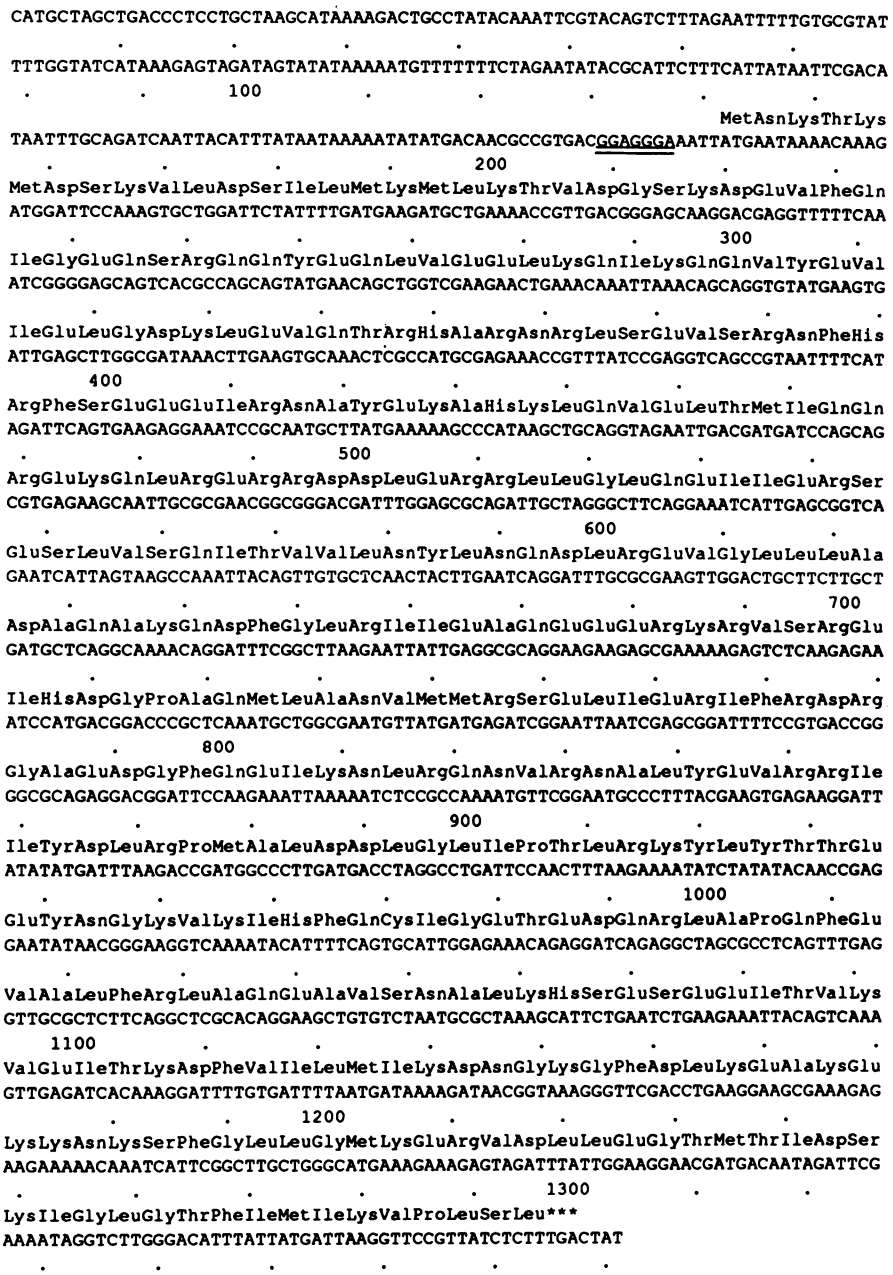


FIG. 2. Simplified restriction map of the DNA inserts in plasmids. The capacity of plasmids containing these inserts to restore levansucrase synthesis to a *sacU* mutant QB254 is indicated. The positions of ORF1 and ORF2 (see Results) are shown (▨). This restriction map and that in Fig. 1 are in opposite orientations. The direction of transcription is from left to right. ORF2 is interrupted in plasmid pBU102 by a *lacZ erm* cassette (not to scale), and ORF1 is interrupted in plasmid pUB103 by an *aphA3* cassette (not to scale) (see Materials and Methods).



maintained at a high copy number in *B. subtilis*(pHV1431d) to produce pBU3. The yield of plasmid pBU3 (50 to 100 μg per liter of culture) compared favorably with that of pBU2 (3 μg/liter). Plasmid pBU14 was a deleted derivative of pBU3 containing a 3.9-kb *Sal*I fragment spanning the *sacU* locus. This *Sal*I fragment contains a 3.6-kb *Sal*I-*Bam*HI segment of *B. subtilis* chromosomal DNA, the restriction map of which was indistinguishable from that of the corresponding segment of phage λ54 (see above). Plasmid pBU16 was derived from pBU14 by eliminating a 1-kb *Sph*I fragment (Fig. 2). This plasmid also restored levansucrase synthesis in the *sacU* mutant QB254. Moreover, both pBU14 and pBU16 led to hyperproduction of levansucrase in the Rec⁻ strain 1A510 (Table 2). These data show that the *sacU* locus, or at least a functional part of it, is located within the 2.6-kb *Sal*I-*Sph*I fragment shown in Fig. 2.

TABLE 2. Secreted levansucrase activities of strain 1A510 harboring recombinant plasmids[a]

Plasmid	Sp act of levansucrase (U/mg × 10^3)	
	Without sucrose	With sucrose
pHV1431d (vector control)	<10	17
pBU14	<10	330
pBU16	<10	670

[a] *B. subtilis* 1A510 harboring the indicated plasmid was grown in MMCH medium supplemented with chloramphenicol and either 1% (wt/vol) glycerol or 2% (wt/vol) sucrose. The culture supernatants of exponentially growing cells were dialyzed overnight against 0.05 M potassium phosphate buffer (pH 6.0), and levansucrase activities were measured. Specific activities were calculated as levansucrase units per milligram of total culture protein.

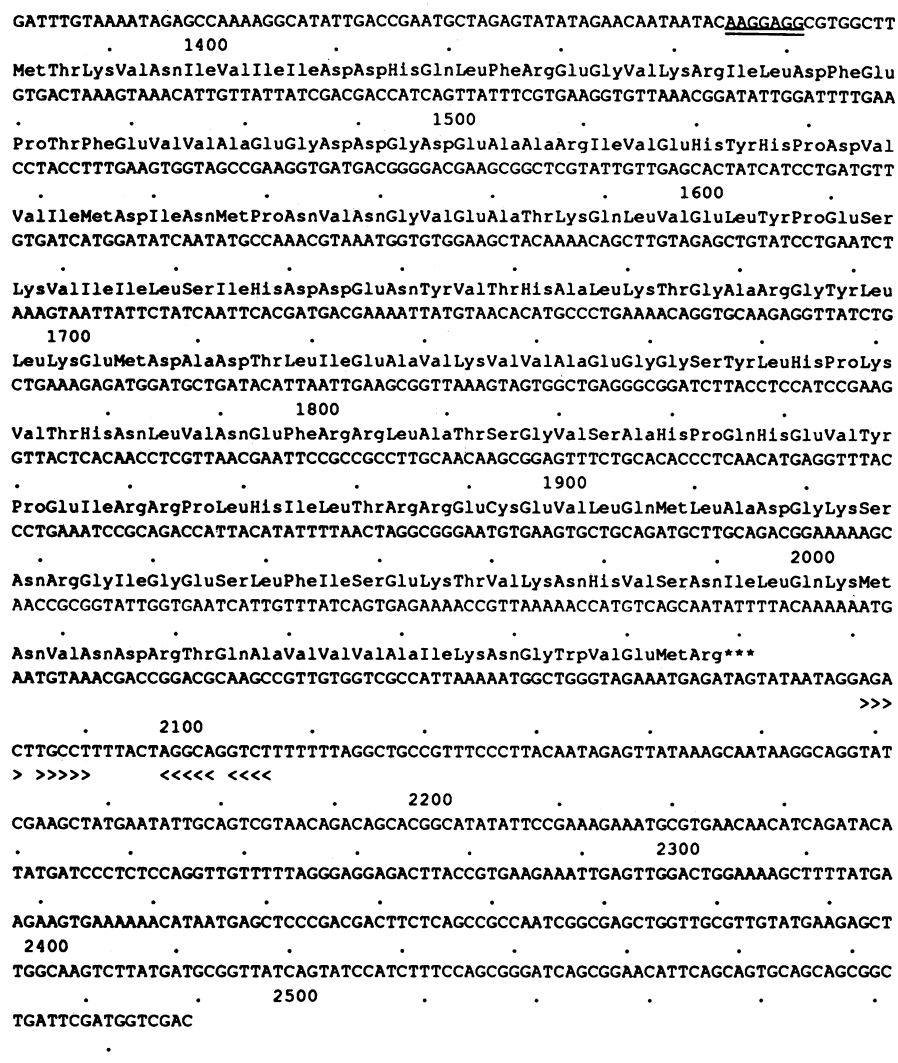


FIG. 3. DNA sequence and inferred amino acid sequence of ORF1 and ORF2 of the *sacU* locus. The ribosomal binding sites are underlined and a potential transcriptional termination signal is indicated by arrowheads.

Genetic fine structure analysis of the *sacU* locus. To locate *sacU* within the fragment inserted in pBU16, deleted plasmids were constructed. Plasmid pBU101 was derived from pBU14 by eliminating a 2.4-kb *Eco*RI fragment. Plasmid pBU100 was constructed by introducing this *Eco*RI fragment into the single *Eco*RI site of plasmid vector pHV1432. Since both plasmids were unable to transform the *sacU* strain QB254 to *sacU*$^+$ (Lvs$^+$) (Fig. 2), we concluded that essential DNA sequences spanned the *Eco*RI site of pBU16. This was confirmed by introducing DNA cassettes into the *Eco*RI and *Bst*BI sites of pBU16, leading to pBU102 and pBU103, respectively. Since pBU14 and pBU16 could not be maintained in *E. coli*, these cassette-containing plasmids were obtained by direct cloning in *B. subtilis*. A 4.5-kb DNA sequence containing *lacZ* and *erm* was inserted at the unique *Eco*RI site of pBU16 to create pBU102. This plasmid was introduced into competent cells of *B. subtilis* 168 by selecting for Em^r Cm^r transformants. Spontaneous Em^r Cm^s integrants arose by homologous recombination (a double-crossover event led to the integration of *lacZ erm* into the chromosome) and loss of the plasmid. Strain QB4210 carrying this *lacZ erm* insertion in the chromosome had a Lvs$^-$ phenotype. Plasmid pBU103 was constructed in a similar fashion by inserting a 1.5-kb *Cla*I fragment containing the kanamycin resistance marker *aphA3* from *Streptococcus faecalis* (49) at the unique *Bst*BI site of pBU16. As above, after introduction of pBU103 into *B. subtilis* 168, Km^r Cm^s integrants arose by insertion of the Km^r marker into the chromosomal *sacU* locus. The inserted marker in this strain (QB4222) again led to an Lvs$^-$ phenotype. The inserted fragments in QB4210 and QB4222 interrupt open reading frames (ORFs) ORF1 and ORF2, respectively (see below).

Nucleotide sequence of the *sacU* locus. The 2.5-kb *Sph*I-*Sal*I fragment of pBU16 was sequenced on both strands by the dideoxy-chain termination method (Materials and Methods). The sequence was examined for ORFs by systematic translation of the codons in the six possible frames. On one strand, stop codons were found regularly in the three possible frames. In the other orientation, we found two successive ORFs, called ORF1 (385 codons) and ORF2 (229 codons) (Fig. 3). These were preceded by strong ribosome-binding sites at optimal distances from the initiation codons,

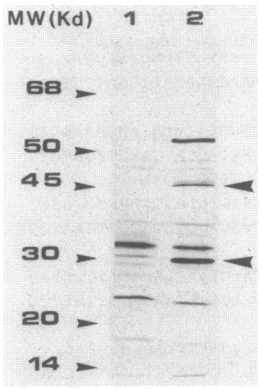


FIG. 4. Identification of the SacU polypeptides. Closed covalent circular plasmid DNA (1 μg) was used in the in vitro assay. Translational products labeled with [^{35}S]methionine were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and detected by autoradiography. Lanes: 1, vector pHV1431d; 2, recombinant plasmid pBU16 containing both ORF1 and ORF2. Migration of standards is indicated in the left margin. Arrowheads in the right margin indicate bands of approximately 45,000 and 30,000 daltons. The 53,000-dalton band, although more intense in lane 2, was also present in lane 1.

respectively, GGAGGGA (ΔG, −78 kJ/mol) and AAG GAGG (ΔG, −74 kJ/mol). No transcription termination signal or obvious promoter sequence was found between ORF1 and ORF2. ORF2 was followed by a 10-base-pair palindromic structure (ΔG, −88 kJ/mol) containing one G-T base pair mismatch and then a T-rich stretch, which may correspond to a transcription terminator (Fig. 3).

The deduced molecular masses of the ORF1 and ORF2 encoded polypeptides are 44,906 and 25,833 daltons, respectively. Both polypeptides have equivalent amounts of basic and acidic residues. Percentages of hydrophobic residues (leucine, isoleucine, methionine, and valine) in the ORF1 polypeptide (29 mol%) and the ORF2 polypeptide (31 mol%) were higher than the value for an average protein (20.2 mol%) (8). In spite of this higher content of hydrophobic amino acids, the hydropathic profiles did not reveal any significant transmembrane segments.

In vitro expression of the *sacU*-encoded polypeptides. The pBU16 plasmid, containing the *sacU* locus, was incubated in vitro with a coupled *E. coli* transcription-translation system (Materials and Methods). The labeled polypeptides were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Fig. 4). Comparison of the polypeptides synthesized by the recombinant plasmid with those encoded by the vector plasmid pHV1431d revealed several additional bands, two of which had apparent molecular masses in agreement with those deduced for the ORF1- and ORF2-encoded proteins (respectively, 45,000 and 30,000 daltons). A small discrepancy was noticed in the case of the ORF2 polypeptide between the experimental value and the one deduced from the DNA sequence. Such differences have previously been observed for other proteins, such as sigma factors.

Comparison of the SacU amino acid sequence with that of known regulatory proteins. A computer search for similarities with other proteins revealed that the ORF1-encoded polypeptide shares homology with members of a class of sensor proteins i.e., *Salmonella typhimurium* CheA, *E. coli* CpxA (Fig. 5), *Agrobacterium tumefaciens* VirA, *E. coli* PhoR, and *Klebsiella pneumoniae* NtrB (9, 34, 39). In the C-terminal portions of these proteins five boxes of conserved amino acids, which we numbered 1 to 5 (ordered from N to C terminus), have previously been described (46). Similarities were particularly striking in a large region spanning boxes 4 and 5 (Fig. 5). However, the amino acid sequence of the ORF1 polypeptide of *sacU* corresponding to box 4 shows only weak homology with the consensus sequence described for gram-negative organisms (46). The *B. subtilis* ORF1 protein and a known sensor protein from *E. coli*, CpxA, show similar degrees of homology with the *S. typhimurium* CheA sensor protein: 14 identical amino acids and 8 conservative substitutions for SacU versus 13 identical amino acids and 4 conservative substitutions for CpxA (*B. subtilis* ORF1 positions 339 through 385, *E. coli* CpxA at positions 410 through 458, *S. typhimurium* CheA at positions 481 through 526) (Fig. 5).

The ORF2-encoded protein shares homology with two classes of regulatory proteins. The *E. coli* proteins OmpR and Dye (10) and the *B. subtilis* proteins Spo0A and Spo0F

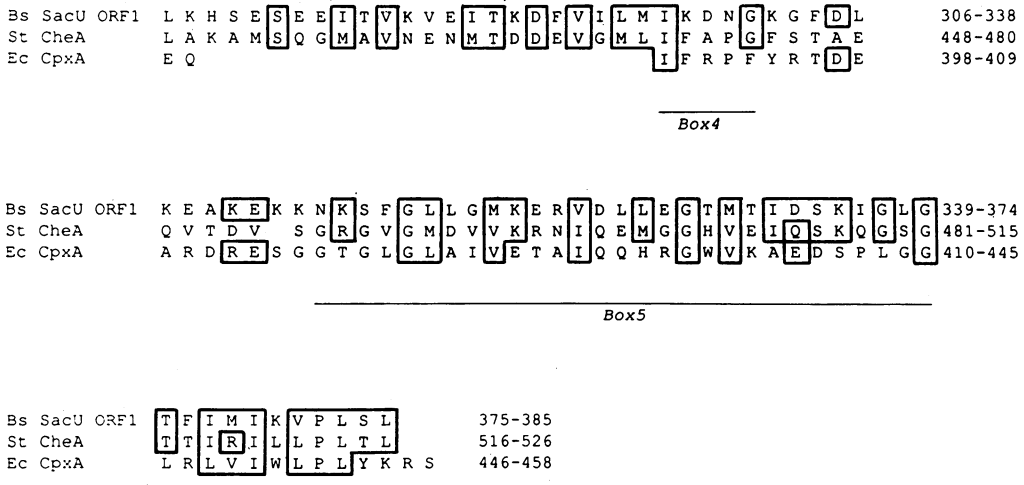


FIG. 5. Alignment of the C-terminal portion of the ORF1 encoded protein of the *sacU* locus with that of a family of sensor proteins. Each polypeptide was individually compared with the *sacU* ORF1 polypeptide, and homologous regions are indicated by boxes. Accepted conservative substitutions: I, L, V, and M, K and R; S and T; D and E; F and Y; N and Q; G and A. Numbers indicate the positions of the residues in the respective protein.

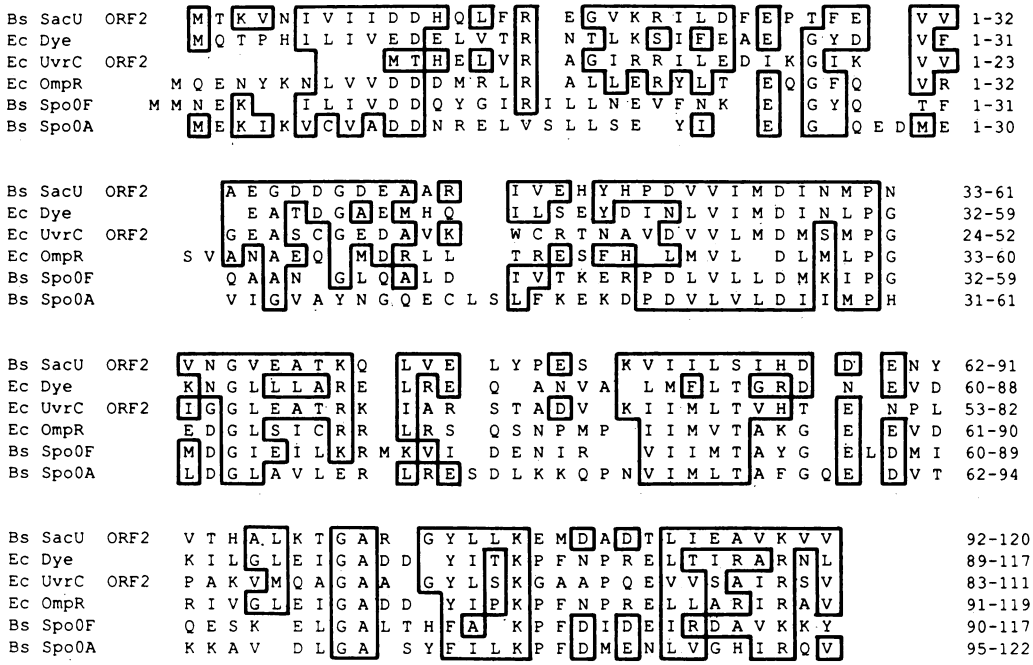


FIG. 6. Alignment of the N-terminal portion of the ORF2-encoded protein of the *sacU* locus with that of a family of regulatory proteins. Comparisons were made as indicated in the legend to Fig. 5.

(12, 27, 39, 48) share extensive homology with the ORF2 protein of the *sacU* locus at the N-terminal domain (Fig. 6). The *E. coli* proteins MalT (6) and the putative positive regulator encoded by ORF1 of the *uvrC* locus (42) share homology with the ORF2 protein at the C-terminal domain (Fig. 7). ORF2 of the *uvrC* locus (42) is a special case, since it shares extensive homology with the ORF2 polypeptide of the *sacU* locus at both the N- and C-terminal portions (Fig. 6 and 7).

DISCUSSION

Two Tn*917* insertions located on opposite sides of the *sacU* region were used to clone the *sacU* locus by chromosome walking with the bacteriophage lambda replacement vector EMBL3 and by direct establishment of recombinant plasmids in *B. subtilis*. In our earlier attempts to clone *sacU* we had tried unsuccessfully to find the locus in a number of *E. coli* plasmid banks. Our present finding that plasmids pBU14 and pBU16 cannot be established in *E. coli* suggests that a DNA sequence or gene product from the *sacU* region may be toxic to this organism.

The DNA sequence of the *sacU* locus reveals the presence of two ORFs referred to as ORF1 and ORF2. No palindromic structure corresponding to a potential transcription-termination signal was found between ORF1 and ORF2. This suggests that ORF1 and ORF2 may form, or be part of, an operon. The ORF2 product appears to be required for activation of synthesis of a class of degradative enzymes in *B. subtilis*, since insertional inactivation of ORF2 led to a levansucrase-deficient (*sacU*) phenotype. The role of ORF1 has not been clearly established. Plasmid pBU100 containing only ORF1 did not restore degradative enzyme synthesis in a *sacU42* mutant, but this can be explained if this mutation lies within ORF2 or lies within ORF1 and is dormant. However, insertional activation of ORF1 also led to a levansucrase-deficient (*sacU*) phenotype. This suggests that the ORF1 product is required in addition to the ORF2 product. Alternatively, insertion of foreign DNA in ORF1 could lead to a polar effect on ORF2 expression. These two hypotheses are not mutually exclusive.

A computer search revealed similarities between the *sacU* ORF1-encoded polypeptide and sensory transducers, especially CheA of *S. typhimurium* (Fig. 5) at the conserved C-terminal portion (34, 46). Likewise, the *sacU* ORF2-encoded polypeptide showed extensive homology with members of a family of transcriptional activators: OmpR,

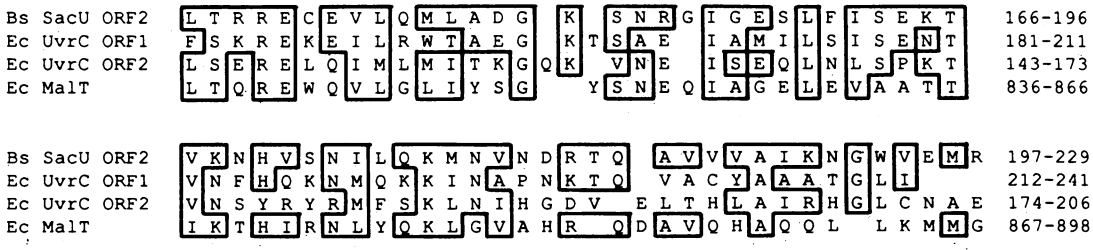


FIG. 7. Alignment of the C-terminal portion of the ORF2 encoded protein of the *sacU* locus with that of a family of homologous regulatory proteins. Comparisons were made as indicated in the legend to Fig. 5.

Dye, Spo0A, Spo0F (Fig. 6), NtrC, PhoB, VirG, and CheY (39). Many of these proteins function in pairs to regulate gene expression at the level of transcription in response to environmental changes such as nutrient limitation (NtrB-NtrC, PhoR-PhoB) or altered osmolarity (EnvZ-OmpR) (29). The ORF1 and ORF2 products of *sacU* may therefore comprise a dual-component system in which the former is a candidate for sensing specific changes in the environment and transducing this information via the latter to the transcriptional apparatus. The conserved N-terminal region of the ORF2-encoded regulator may interact either with the sensor protein or with the transcriptional apparatus, conferring an antitermination or transcriptional activation function. Alternatively, this putative function may be located in the conserved C-terminal domain, since ORF2 shows homology with positive regulators such as *MalT* or *E. coli*. Similar results concerning homologies between the products of the *sacU* locus and a series of sensors and regulators have been obtained by Henner et al. (18).

Different types of interactions between sensor and regulator proteins have been proposed: direct protein-protein contact in the case of the EnvZ-OmpR couple (29) or phosphorylation of the regulator in the case of the CheA-CheY and NtrB-NtrC couples (33, 50). It has been shown that the CheA sensor protein undergoes autophosphorylation in the presence of ATP to produce phospho-CheA (19, 35, 50). The phosphorylated sensor protein then donates its phosphoryl group to the CheY regulator in a way which may be comparable to the phosphotransferase reactions of the phosphoenolpyruvate-sugar phosphotransferase system (50).

Concerning the organization of the *sacU* locus, the genes encoding ORF1 and ORF2 may be part of the same operon, as in some other two-component systems (*ntrB ntrC*) (34). However, this may not be a general rule, since *cheA* and *cheY*, for instance, map at different loci in *S. typhimurium* (30).

The *sacU* system affects the production of enzymes that degrade polymeric carbon and nitrogen sources (e.g., levansucrase, α-amylase, β-glucanase[s], proteases). One may speculate that the signal transmitted to the sensor protein reflects carbon and/or nitrogen limitation. No obvious transmembrane segment could be detected in either of the two *sacU*-encoded polypeptides. Thus, the *sacU* system may respond to an intracellular signal of nutrient limitation. Two *sacU*(Hy) mutations have been sequenced (18) and shown to be missense mutations in the amino terminus of the presumed regulator protein (ORF2 product). It will be informative to study the interactions between the *sacU* products and those of the *sacQ* and *prtR* genes, which encode small regulatory polypeptides, which also affect the rates of synthesis of the same class of degradative enzymes.

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ADDENDUM IN PROOF

The *sacU* ORF2 sequence was reported independently by T. Tanaka and M. Kawata (J. Bacteriol. **170**:3593–3600, 1988). *sacU* ORF1 and ORF2 will be renamed *degS* and *degU*, respectively (see also reference 18).

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